artment of Agriculture Agricultural Research Service January 1989

Agricultural Research

1989
Year of the Bee?

How Reliable Are the Numbers?

With all of the super-sensitive instrumentation and sophisticated analytical techniques available to modern science, one might think that published values on the solubility of pesticide chemicals would be matters of scientific fact—tested and proven.

Take, for example, the relevant data for alachlor, a pesticide chemical that's been widely used for 20 years. Alachlor's solubility is a critical factor in evaluating the likelihood of its moving inadvertently from croplands into our water supplies.

Yet, according to one reference manual for chemical structures and properties, 140 milligrams of alachlor will completely dissolve in 1 liter (1,000 grams) of water at 23°C while the tables in another manual rate alachlor's solubility as 242 mg/L at 25°C.

The warmer water specified in the higher value can be discounted. It might have caused a few more milligrams to dissolve, but it hardly explains a difference of nearly 60 percent.

Which value is correct, or are both wrong? And why is there such a difference?

Solubility discrepancies are not isolated cases. Published figures on mineral levels in foods also contain inconsistencies. Take selenium: long recognized as an important trace mineral in the human diet, selenium has recently become the subject of increasing scientific interest because of its possible role in cancer prevention. But selenium levels in raw beef, for example, have been variously reported as low as 5 and as high as 40 micrograms (millionths of a gram) per 100 grams.

The more we examine the literature, the more convinced we are that shaky and questionable data are routinely accepted and promulgated as scientific fact. Something must be done—and not just to save professional reputations and institutional credibility. Our courts, legislatures, and regulatory agencies are increasingly guided by scientific studies using elaborate computer models to simulate potential courses of events. Several models for predicting the environmental effects of pesticides are already in use. If the data going into these models aren't reliable, then what are we to make of the data coming out and the decisions based on them?

At ARS, we're creating computer programs of the expert systems type to assess the quality of data in selected fields of research. A program to evaluate data on selenium levels in foods is already in use by the agency's Nutrient Composition Laboratory in Beltsville, Maryland. Another program for data on the solubility of pesticides is being

(Continued on page 13)

Letters

We invite letters from readers and, from time to time, will share them in this column.—Ed.

On Bean Plant Diseases: The article on Priming Beans Against Rust in the September issue does not tell the whole story since nothing is said about the very important contribution made by ARS/Germplasm, especially the Tropical Agriculture Research Station at Mayaguez, Puerto Rico and the long-term bean projects responsible for collecting, evaluating, improving and releasing the lines from which Dr. Stavely's bean rust resistant lines derive a great part of their resistance genes.

The uniqueness of this germplasm, in addition to its tropical origin, is that the original collection, Mexico 309, contained a series of resistance genes closely positioned (linked) on a single chromosome so that they could be handled in breeding as though they were a single major gene, thus greatly simplifying passing the resistance on to subsequent lines.

I do hope that in future articles your writers will delve more carefully into the background behind each story.

George F. Freytag, Research Geneticist
Mayaguez, Puerto Rico

Author Stephen Berberich replies: Thank you for your comments on my article and for suggesting additional information to our readers. Dr. Stavely and I discussed the Mexico 309 and other germplasm sources for his super-rust resistant bean lines, as well as the contributions of many other scientists. However, it was not my intent here to tell the whole story of how science produced the bean lines. Instead, I hoped to attract the interest of potential users of this new biological technology—seed companies, other breeders, bean growers—to speed these experimental beans to our dinner tables.

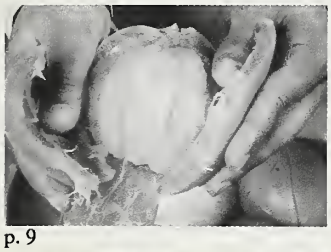
On FIFRA: The Forum page of *Agricultural Research*, September 1988, on Biologicals Favored in Crop Protection Plans contained an error.

The Federal Insecticide, Fungicide, Rodenticide, and Acaricide Act (FIFRA), not the Delaney Amendment, requires the U.S. Environmental Protection Agency to reregister pesticides. The process began in 1974 and will continue into the next century. FIFRA is a risk benefit law and does permit the use of some pesticides known to be weak carcinogens in some test animals. Only two widely used nematicides, EDB and DBCP, have been cancelled by EPA.

Barry M. Brennan, Pesticide Coordinator
Manoa, Hawaii

Agricultural Research

Cover: Starting from an accidental release near Sao Paulo, Brazil, in 1956, African-European hybrid honey bees have spread over most of tropical and subtropical South America, all of Central America, and now within 500 miles of the United States' southern border. Cover design by Sandy Henry, ARS.



p. 9

p. 4

p. 14

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Agricultural Research Service

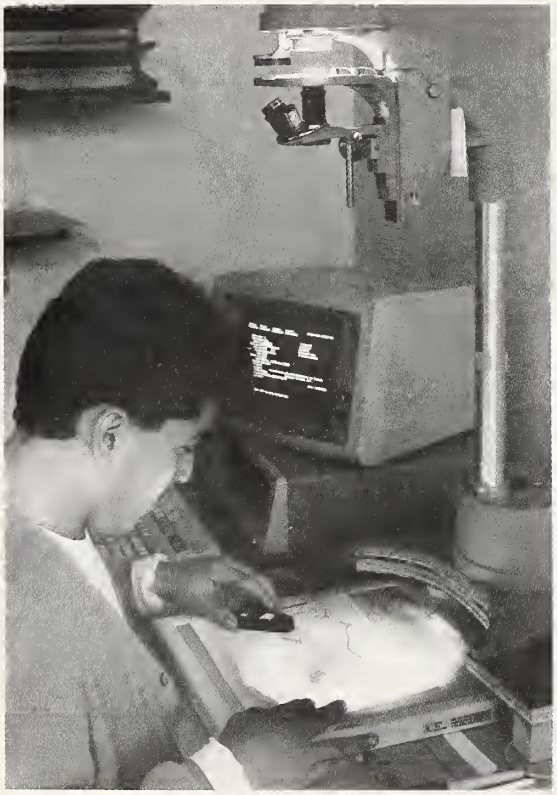
Year of the Africanized Bee?

To take a quick break, Dom Martinez left his station counting shipping cargo on the state-run docks at Mobile, Alabama, and stepped outside the warehouse. He looked up at a wall and saw about 200 bees.

Since officials are always checking the docks for imported insects, Martinez called the local office of USDA's Animal and Plant Health Inspection Service.

Two minutes later, Plant Protection and Quarantine Officer Glen Landau, who was doing a routine ship inspection not far away, got a call over the radio attached to his belt. The message: possible Africanized bee infiltration.

Within 5 minutes, he was there. He took his bee suit, insecticide, and nets out of the back of his car and suited up for action.



DAVID NANCE

Juan Aranda, an ARS student trainee at Weslaco, Texas, scans magnified images of honey bee body parts into a computer. The computer then calculates the probability that the bee was Africanized. (88BW2140-34)

As had happened the previous 8 times Africanized bees were suspected to be in Mobile, Landau collected 100 of the bees in his net and killed the rest with spray—in case they were Africanized. The difference between this case and the others, however, is that this time—September 26, 1988—Africanized bees *had* found their way into the United States. Over the next few weeks, Landau and colleagues would set up traps in a 2-mile radius of the docks and alert beekeepers in a 10-mile radius of the infiltration—all to be on the lookout for other bees.

But how did he know these were Africanized bees and not the average domestic European honey bees already here? After all, the bees look the same.

He knew because he had sent the 100-bee sample, preserved in alcohol, by overnight express parcel service, to the Beneficial Insects Laboratory in Beltsville, Maryland. That laboratory, part of the Agricultural Research Service, provides expert identification of Africanized bees 24 hours a day, 7 days a week, including holidays.

At the lab, Steve Sheppard and Robyn Glass used FABIS (for Fast Africanized Bee Identification System) to check the sample. FABIS was developed by ARS' Thomas E. Rinderer and colleagues at the Honey Bee Breeding, Genetics, and Physiology Laboratory in Baton Rouge, Louisiana.

They mounted the forewings of 10 randomly selected bees on slides and projected them, enlarged, onto a screen. They measured the wings and checked the results against a chart of standard wing specifications for each kind of bee. The result: probably Africanized.

So they went on to step 2: a complete morphometric (body measuring) analysis. They measured forewings, hind wings, hind legs, and abdominal sternums in many different places and angles, for a total of 25 separate measurements. Then they entered all the data into a computer, which gave them a figure indicating probability of Africanization—in this case, 99.4 percent. "That's pretty close to a definite yes," Sheppard says. He alerted Landau that the bees were Africanized.

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Practically indistinguishable from their European cousins, Africanized honey bees are noted for savagely defending their hives.

The scientists at the lab have a research plan to develop new methods of distinguishing between the two kinds of bees—methods that analyze molecular, chemical, and immunological differences.

In fact, ARS has 4 locations with 11 scientists conducting research on the Africanized bee. The goal of that research: to stop or slow the spread of the bee northward into the United States from Mexico and if that's not possible, to learn how to cope with it.

Research Gone Awry

The Africanized bee situation can be traced back to a research project that went awry. In 1956, a Brazilian geneticist imported African varieties of *Apis mellifera* and bred them with European varieties in Brazil. His purpose: To improve tropical honey production by creating a honey bee well suited to hot climates.

But the experimental colonies were accidentally released before the geneticist could assess the hybrid bee's characteristics.

Unfortunately, those characteristics, according to scientists at the ARS Baton Rouge lab, include less honey

production and less efficient pollination. Research showed that compared to European bees, Africanized bees collect nectar with less sugar, carry smaller loads, make longer trips, and don't communicate as much with fellow bees about good nectar locations.

Since these bees threaten to come to the United States, beekeepers and farmers fear for their businesses. And with reason: Bees produce $150 million worth of honey and pollinate $20 billion worth of crops every year.

But perhaps more frightening to people is the Africanized bees' reputation for stinging in greater numbers and with less provocation than European bees. Although their venom is no more poisonous than that of their European counterparts, the greater number of stings can lead to shock and possibly death in a victim.

"People sometimes refer to these bees as more aggressive, but that's not really an accurate term. What they are is more defensive."

Thomas Rinderer, ARS geneticist, Baton Rouge, Louisiana

When provoked, the bees will also chase a suspected hive molester a lot farther—up to a mile; the European type generally gives up after a few dozen feet.

"People sometimes refer to these bees as more aggressive, but that's not really an accurate term," says Rinderer of the Baton Rouge lab. "What they are is more defensive." He explains that the bees are simply defending their hive. European bees do so, as well, but not as fiercely.

And he adds, there is some good news to the story. Interbreeding with native European populations has made each generation of the Africanized bees gentler. "The bees in Mexico are not the same as the ones in Brazil and certainly not the same as those in Africa." What that means is that the

JACK DYKINGA

ARS plant physiologist Gerald Loper adjusts a bee trap that will be suspended from a balloon tethered 25 to 50 feet above the ground. Once the drones have been drawn near by a synthetic queen bee pheremone, cigarette filters dyed to look like queens lure them into the trap. Radar trailer in background is used to track groups of drones in flight. (0587X431-24)

more desirable characteristics of European bees have softened the negative ones of the original Africanized hybrids.

USDA officials are taking full advantage of this definite—albeit slow—tendency to change genetically with interbreeding. Two USDA groups about 500 miles south of the U.S. border—at Veracruz in the east and Oaxaca in the west—have been importing and releasing gentle European bees to interbreed with Africanized bees there. They also trap swarming bees in bait hives and destroy them with suffocation by sealing the hive in a plastic bag.

ARS research at the Baton Rouge lab contributed to the knowledge necessary to implement the program, and scientists there continue to support control efforts.

The two units started as a project between the Mexican government and USDA to trap and kill the bees in 1986. That has slowed the bees some; by expert projections, they could have arrived in south Texas in 1987 or 1988, Rinderer says. But they're still 500 miles south of the border.

In case the bees do come to the United States, ARS scientists hope to help beekeepers and the public be ready. Research to do this follows.

Stopping and Controlling Africanized Bees

H. Allen Sylvester and colleagues at the Baton Rouge lab are "mapping," or identifying and locating, the genes of European bees. "We want to genetically engineer a strain of the more gentle domestic bees that will outcompete the Africanized bees in some way," Sylvester says. For example, a bacterium called *Bacillus larvae* causes one of the worst honey bee diseases, called American foul brood. The scientists hope to find a way to modify European honey bees to produce a bacterium-killing protein called cecropin (sakropin). The gene that allows insects to do this has been identified in a moth; now Sylvester and Rinderer are working to find a way to get that gene into honey bees.

Then, beekeepers would have the bacterium-resistant strain in their hive yards and could set out honey baits with foul brood spores mixed in. The honey would attract any honey bee that comes along into eating it. The genetically engineered European ones, because of their new gene, would kill the bacterium hidden in it, but nonresistant Africanized bees would die.

WILLIAM RUBINK

Bait hives are assembled by Mexican Department of Agriculture technicians near Ciudad Victoria in eastern Mexico. The hives provide nesting places for Africanized bees that will later be destroyed. (88BW1395)

Rinderer says his group is also working on finding natural and synthetic compounds to subdue the bees. One winner: a mosquito repellant developed by ARS in the 1950's, called Deet, which is now in more than 30 insect repellants on the market. Deet quickly subdues bees in lab tests. Although the bees eventually recover, Deet makes them stop stinging completely at the moment, giving a victim time to run away. "It's kind of like Mace in that it temporarily debilitates them," Rinderer says.

He points out that Deet would have to be sprayed in the air near the person or animal being stung. In tests, spraying the compound directly on the skin before the attack was not as effective at subduing the bees as was permeating the air with it at the moment of attack.

For national parks and other outdoor public areas, the scientists have developed a system for trapping and killing

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Africanized bees are likely to swarm several times a year, accounting for their rapid propagation. This swarm, near Tapchula, Mexico, was destroyed.

Africanized bees in a way that is environmentally sound. Officials could put out a sugar syrup bait and check to see what kind of bees have responded to it, Rinderer says. If Africanized bees are

there, they would put a couple of drops of poison in the syrup to kill the bees. "Since there is no spraying, risk to the environment is minimal," he says.

Detecting and Attracting Bees

At the Carl Hayden Bee Research Center in Tucson, Arizona, scientists are tracking bees with radar as they search for mates to learn exactly how far and where a queen goes to find a group of males. They hope to fill beekeepers in on how far and in what direction a queen flies to mate. "That way, a beekeeper could check her probable destination for Africanized males before letting her fly to mate," says Gerald Loper. If Africanized males are there, the beekeeper could replace them with European males.

Also, by learning where males gather to mate, areas where Africanized bees may spread could be predicted so that their arrival could be anticipated and they could be destroyed.

Another project is developing the best hives for trapping swarms of Africanized bees. Scientists have worked out exactly which odors attract these hybridized bees and how much room they like when they select a new nest. If Africanized bees arrive, these custom-made hives could be set out to trap bees and monitor areas for spread. If Africanized bees do move in, they could be destroyed.

William Rubink, of the Honey Bee Research Laboratory in Weslaco, Texas, has set up three lines of traps, each 115 miles long, in northern

(Continued on page 11)

Can't Tell Your Bees Apart? Bar Code 'Em!

Bees in Tucson are sporting a few extra stripes these days—bar codes like those found on packaged foods and other merchandise.

They are the world's smallest bar codes. Nine stripes, less than one-tenth an inch long, are glued to the tiny hairs on bees' backs at the Agricultural Research Service's Carl Hayden Bee Research Center in Tucson, Arizona. An electronic bar-code reader at the doorway of the beehive records each bee's exit and entrance.

Scientists hope the system will reveal such things as how hard honey bees work collecting pollen and nectar, the number of trips each bee makes, the length of time each spends foraging, and how resistant bees are to pesticides.

"Keeping track of individual bees used to be almost impossible because they all look alike," says entomologist Stephen L. Buchmann. "We couldn't easily monitor bees' leaving and returning to their hives. Now we're keeping a dossier on each tagged bee to record its activity over a long period."

The research findings, kept on a computer for easy analysis, might yield new clues to selecting healthier and more productive bees as parents for future generations of honey bees. Without the electronically read bar codes, research on the often mysterious behavior of honey bees takes an enormous amount of time and excessive handling of bees.

Buchmann got the idea of using bar codes from visits to the local supermarket. Bar codes are found on most packaged foods to keep tabs on product costs and to monitor inventory. In the 1960's, ARS helped pioneer commercial application of bar codes for supermarket use.

Buchmann's challenge was to reduce the postage-stamp-size, or larger, bar codes to a small rectangle less than the width of a bee's back. He succeeded by lowering the typical number of bars from as many as 55 to 9 because he only needed to keep tabs on 100 bees at a time. Standard bar codes can differentiate more than a million items.

DAVID RING

Weighing less than 20-millionths of an ounce, the bar code glued to this worker bee lets researchers automatically monitor the bee's passage through the hive entrance as it goes for nectar and pollen. (88BW2061-11)

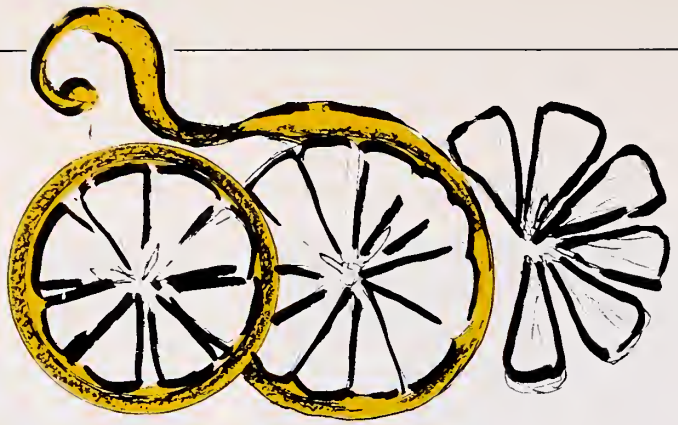
Previously, Buchmann marked bees with dyes and paints that often wore off. Other systems required painting a white dot on bees' backs, then hand-painting a number over it after the dot dried.

Not only was this time-consuming, it also involved much handling of the bees, which upset their normal behavior and flawed research findings.

Buchmann says, "Our bar-code paper and glue weighs about one-twentieth as much as the nectar and pollen carried on each foraging trip away from their hives. So the bees behave normally, unaware of what's on their backs."

Buchmann is working with Sprague Ackley, a researcher with INTERMEC Corp. of Lynnwood, Washington, developers of bar codes and scanning equipment.—By **Dennis Senft,** ARS.

Stephen L. Buchmann is in the USDA-ARS Carl Hayden Bee Research Center, 2000 East Allen Road, Tucson, AZ 85719 (602) 629-6327. ♦



A-peeli Citrus Te

Ever wonder what that white, fleshy stuff is that sticks under your fingernails when you peel an orange? It's the albedo, the bitter-tasting tissue that holds the outer peel to the fruit.

Dissolving the albedo would make it easier to peel citrus because there would be nothing to hold the peel to the fruit. That's the idea behind a patented U.S. Department of Agriculture process that will make it easier for the citrus industry to produce orange, grapefruit, and other citrus sections.

The process, called vacuum infusion, uses a vacuum chamber to remove air from inside the fruit. Once the vacuum is released, an approved food enzyme is sucked into the air spaces and breaks down the albedo.

"Citrus processors can use this new technology to make a variety of consumer products, such as ready-to-eat fresh oranges and grapefruit and fruit sections," says Robert A. Baker, a chemist with USDA's Agricultural Research Service.

Vacuum infusion injects pectinase enzyme into the gas spaces between the peel and fruit. "It may seem odd, but there are 100 to 200 milliliters of gases such as carbon dioxide, nitrogen, and oxygen in a grapefruit," Baker says. "That's about enough to fill a tennis ball."

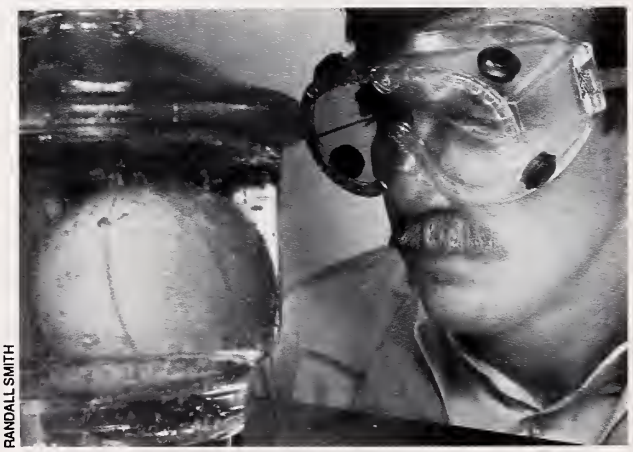
Once the albedo is dissolved, scientists can easily pull off the peel without having it stick to the fruit surface, says Baker, based at the ARS Citrus and Subtropical Products Laboratory in Winter Haven, Florida. The process was patented by Joseph Bruemmer, a chemist at the lab.

"Citrus processors could use this technology to make it easier and faster for workers to remove the peel and pull apart the sections by hand," Baker says. "And mechanical peeling machines would be much more effective after the peel is loosened."

The process may also have economic benefits. Workers now peel and section citrus by hand, and from 30 to 40 percent of the fruit and juice is lost. He says vacuum infusion could improve fruit quality, lower production costs, and offset fruit section imports from Mexico, Taiwan, and other countries where cheap labor is available.

About 10 citrus companies have asked for information on the technology and at least one processor in Florida is seeking a patent license to develop the technology.

"For this to be commercially feasible, it would have to be done continuously, like an assembly line," Baker says. "We do several fruit at a time in batches in the laboratory, but the rate



RANDALL SMITH

In his Winter Haven, Florida, laboratory, chemist Robert Baker watches grapefruit undergoing first step of vacuum-infusion peeling. (88BW1769-23A)

g New hnology

would need to be increased for commercial production."

Cheaper imported citrus sections and the labor intensiveness of the process have led to a decline in sectioning in Florida. Between 1972-73 and 1982-83, production of Florida grapefruit and orange sections fell from 2.8 million boxes to just over 1.4 million. In the 1970's, there were about a dozen sectioning plants, but now only two remain.

But the new technology could help turn things around, Baker says. "The process produces clean, high-quality orange and grapefruit sections that can be placed in jars, canned, wrapped in plastic and sold as ready-to-eat peeled fruit, or used in salad bars. These are popular with consumers who want easy-to-prepare or ready-to-eat products."

Vacuum infusion works like this: an orange or grapefruit peel is scored with a knife about six times and submerged in a container of pectinase in water. Pectinase, an approved food enzyme used in the jelly and jam industry, breaks down pectin in the albedo. It's a dilute solution—about 200 parts per million of pectinase.

The container is then put into a vacuum chamber for 2 to 3 minutes. This draws the gases out of the fruit, causing bubbles to flow from inside the peel, via the score marks, through the solution.

Once the gases are drawn out, the vacuum is released and the solution is sucked into the spaces where the gases were. Then the fruit is left submerged in the solution for about 15 minutes to 1 hour, depending on the temperature of the fruit and concentration of the enzyme. "This gives the pectinase time to dissolve the albedo," Baker says. "The enzyme doesn't break down the fruit itself or the membranes that surround the sections."

After this time, the peel can be removed. "It almost falls off, because the cells of the albedo no longer adhere to one another. And it leaves the inner core of fruit clean—much cleaner than if you peeled it yourself."

There is little, if any, loss of edible fruit or juice in the process, Baker says, compared to the 30 to 40 percent losses when fruit is cut up into sections.

Because the pectinase solution is so dilute, it has no taste, and fruit flavor is not affected by it. The enzyme itself, approved for food use by the U.S. Food and Drug Administration, is inexpensive. He estimated it would cost about one-tenth of a cent or less per grapefruit, depending on the price and availability of the enzyme.—By **Sean Adams,** formerly ARS.

Robert A. Baker is at the USDA-ARS Citrus and Subtropical Products Laboratory, P.O. Box 1909, Winter Haven, FL 33883 (813) 293-4133. ♦

RANDALL SMITH

Peel practically falls off grapefruit treated by an approved food enzyme in an ARS-patented process. (88BW1775-2)

Innovation in Foods: Safety First

Now that the world has come to accept "ham" and other meat delicacies made from poultry rather than pork and beef, another prospect looms on the horizon: Would you believe fish franks?

Actually, the idea is not so farfetched. But how safe would it be? Scientists have already begun pondering the potential safety of adding minced fish or surimi, a paste made from minced fish, to partially replace meat in cured products. The work is being done at the Eastern Regional Research Center operated at Philadelphia, Pennsylvania, by the U.S. Department of Agriculture's Agricultural Research Service.

The research has been requested by USDA's Food Safety Inspection Service (FSIS), which has already received industry queries on permitting the use of minced fish in meat products such as frankfurters, according to Walter Fiddler, a research chemist at the ARS center.

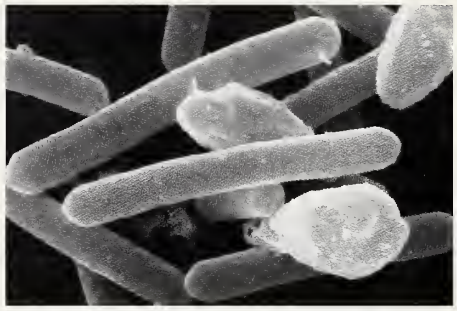
"Industry wants to add the fish for nutritional quality, binding, and gel characteristics," says Fiddler. "The United States has a significant amount of underutilized fish—either whole species or ones where just fillets are used and there are parts left over."

The addition would be an obvious economic boon for the domestic fish industry. The U.S. sausage market, excluding poultry items, totaled about 5.04 billion pounds in 1981, almost twice the size of the U.S. market for fish. With a mere 5 percent minced fish added to meat franks alone, demand for fish flesh would rise some 45 million pounds.

But there are health concerns about adding the fish to frankfurters and their culinary kin, Fiddler notes.

"Nitrite is used in the curing of meats, and it performs a number of important functions," he says. "It gives the cured meat that stable pink color, it imparts the characteristic flavor of cured meat products, and it helps keep the fat from getting rancid. Its most important property is that it prohibits the growth of *Clostridium botulinum,* which causes botulism.

"But fish is high in chemical compounds called amines, and nitrite



BEVERLY MALEEFF

***Clostridium botulinum,* a toxin-producing food spoilage organism and dangerous human pathogen. The sac-like dormant spores can survive conditions that are lethal for the rod-shaped bacterial cells. Magnified about 8,500 times. (88BW2145)**

combines with these amines to form nitrosamines. This combination can occur during processing or in the smokehouse, when the temperatures may go up to 150°F. Heat favors this reaction.

"Although these nitrosamines are found in very low concentrations, parts per billion, some of the ones that have been tested have been found to be animal carcinogens."

Fiddler's study, begun in 1987, is focusing on such factors as harvest time of the fish, the species used, the age of the fish before it's processed, and the length of its stay in frozen storage before processing.

"We want to know how these affect the nitrosamine level of the product after processing and after home cooking," Fiddler says. "There seems to be a lot of industrial interest in the use of surimi; the fish industry is looking for a higher value-added product. This may lead to a new generation of meat products."

Protein and Iron Source

Another possible meat product additive being studied at the Philadelphia center is the blood that normally goes down the drain at slaughterhouses. As with the extra fish parts, the blood currently offers no income for the meat industry and even poses a problem in waste disposal.

"A number of companies have petitioned FSIS to incorporate some of this blood in cured meat products," says Arthur J. Miller, a food technologist at the laboratory. Consumer studies at Louisiana State University at Baton Rouge have shown meat products with up to 2 percent blood content are acceptable.

"It boosts the nutritional value of the meat product, increasing its protein and iron content."

But once again, *C. botulinum* may complicate matters. "It also needs iron to grow," says Miller. "In cured meat products, the nitrite chemically binds the iron inside *C. botulinum* cells and poisons them; that's how it kills the bacterium. But if you have a lot of iron floating around outside the cell, nitrite will bind with that iron rather than enter the cell, so the bacterium may survive.

"We've made up sausages with nitrite and the blood, and preliminary indications are that high iron will render the nitrite ineffective against the bacteria. We're looking at using red blood cells, whole blood, or plasma at different percentages."

The outcome of this study, begun last summer and expected to continue through the spring of 1989, "will provide FSIS with information to help them decide whether to permit the blood as an additive in these meat products," Miller says.

Irradiation To Control Bacteria

The scientists at Philadelphia are not only concerned about what goes into food. They're equally interested in what comes out of it nutritionally. Studies have shown that while high enough levels of irradiation will kill all bacteria and spores of pathogens such as *C. botulinum* in food, the process can affect the vitamin content of certain foods.

"Since 1985, we've set up a program on low-dose irradiation of pork chops and chicken breasts," says Jay B. Fox, Jr., a chemist at the Philadelphia center. "We covered a range of temperatures and doses of irradiation to encompass projected commercial processes for irradiation pasteurization treatments of meats and poultry."

"We went from –20°C to +20°C and from 0 to 7 kiloGrays of irradiation. One kiloGray is equivalent to the

absorption of 0.43 BTU of energy per pound. Our interest was in vitamin loss, specifically vitamins such as riboflavin, thiamine, niacin, B12, and B6.

"The B vitamins in pork were especially sensitive to irradiation, particularly thiamine. But in chicken, irradiation caused very low or negligible loss of vitamins."

"Just by itself, thiamine is exceedingly sensitive to irradiation; fractions of a kiloGray will destroy it. But when you put the thiamine in a piece of meat, it takes a hundred times as much irradiation to destroy it. We want to determine why this occurs with thiamine, and also with riboflavin and niacin under irradiation. If we knew more about the conditions which destroy these vitamins, we could control that destruction."

About 32 countries have approved irradiation of more than 40 foods, and the United States was among nations approving a United Nations accord in 1980 that said any food irradiation dose up to 10 kiloGrays would be considered safe.

But use of irradiation as a food preservative in the United States has been minimal. In 1964, the U.S. Food and Drug Administration approved irradiation of potatoes to inhibit sprouting, and spices may be irradiated at doses of 10 to 30 kiloGrays to kill insects and control microorganisms. The FDA in April 1986 adopted approval of irradiation of all fruits and vegetables up to 1 kiloGray, and papayas irradiated with 1 kiloGray to kill insects have been test-marketed. Consumer acceptance of the papayas was reportedly favorable.

In addition, the FDA in July 1985 approved the use of doses of irradiation from 0.3 to 1 kiloGray in pork to control trichina, the worms that cause trichinosis. However, FSIS is still drawing up the procedures by which pork processors can use irradiation.

"We're interested in the compounds that might be produced in meat by irradiation and whether they could be used as markers to determine if the food has been irradiated. Most of the compounds produced by irradiation are the same as those produced by cooking meat—but not all of the products of irradiation have been identified."

Eventually, ARS hopes to provide information on the likelihood of bacterial threats directly to food processors by means of a computer program now being developed at the Philadelphia center.

That effort, begun in 1987, focuses on "a combination of food safety problem organisms," says Robert L. Buchanan, a microbiologist and research leader at the laboratory's Microbial Food Safety unit.

When the project is completed, processors will be able to type in a simple set of variables—product temperature, pH, salt concentration, nitrite concentration and whether the processing is done with or without oxygen. In response, they'll get a quick answer on the potential growth rate of any of six pathogens in question.

Buchanan says the initial model for one of the pathogens, *Listeria monocytogenes*, could be complete in 3 to 4 months. The other pathogens are *Shigella flexneri, Aeromonas hydrophila, Salmonella typhimurium, Staphylococcus aureas*, and the ever-worrisome *Clostridium botulinum.*—By **Sandy Miller Hays,** ARS.

Robert L. Buchanan, Walter Fiddler, Jay B. Fox, Jr., and Arthur J. Miller are at the USDA-ARS Eastern Regional Research Center, 600 Mermaid Lane, Philadelphia, PA (215) 233-6620. ♦

Africanized Bees

(Continued from page 6)

Mexico and southern Texas. Located along the Africanized bees' predicted corridor of travel into the United States, the traps are baited with a chemical that lures both types of bees.

The traps will do two things: Let scientists know if and when Africanized bees infiltrate the area and in what quantity, and provide information about existing European populations in the area.

Monitoring European bees now will tell scientists if Africanized bees spread bee parasites and if they change the native bee's body size and swarming behavior. That will give officials in other areas advance warning of what to expect and how fast.

The officials in Mobile, Alabama, haven't had any more Africanized bee trouble, with the possible exception of a couple of stragglers that escaped Landau's spray. The day after Landau collected his sample, a stevedore who works for the same company as Martinez "got the stragglers with a broom and gave them to me," Landau says. Fortunately, beekeepers and the public can rely on research—not brooms—to ready them for the arrival of the Africanized honey bee.

"Honey Bees Abroad"

There's renewed demand for this short brochure, originally intended as a training aid for U.S. civilians and military personnel going overseas. Today, it's proving to be relevant reading for the thousands of Americans who wonder if they'll be affected by an immigration of Africanized bees.

Brief and to the point, the brochure is based on the practical experiences of bee researchers. It tells how to deal with the sting-prone honey bees found in tropical Africa, Asia, and Latin America. Send for your copy of USDA/ARS Program Aid 1425, Honey Bees Abroad, available in limited quantities from the USDA Office of Information, Washington, DC 20250-1300.—By **Jessica Morrison,** ARS.

[If you are interested in contacting scientists mentioned in this article, write or telephone the Editor, Agricultural Research, Bldg. 005, Beltsville Agricultural Research Center-West, Beltsville, MD 20705 (301) 344-3280.] ♦

Milk Protein Mystery Finally Solved

A long-standing mystery about the molecular structure of casein—the major protein group in milk—has been solved, according to three scientists with the Agricultural Research service.

And the solution, they say, could help producers of cheese and some dry-milk products develop more efficient and reliable processing methods.

"We now have an excellent picture of how these proteins are structured at the molecular level and how they actually interact with water," says one of the trio, Helmut Pessen of the ARS Eastern Regional Research Center in Philadelphia, Pennsylvania.

The picture was developed by Pessen, Thomas F. Kumosinski, and Harold M. Farrell. All three are chemists with the Philadelphia research center.

Because casein molecules appear to combine in a random way, Pessen says, their molecular structure has been a matter of scientific speculation ever since the protein was first investigated nearly 60 years ago. A prominent theory in recent years held that casein molecules naturally congregate into particles having no definable or uniform structure. Each of the particles is coated with water, so the theory goes, and won't stick to another until this bound water is somehow removed.

"We found that casein was truly unique. Its molecular structure is very loose, and it contains at least 10 times as much water as any other protein."

Helmut Pessen, ARS chemist, Philadelphia, Pennsylvania

But there are problems with this theory, says Kumosinski.

"For one thing," he points out, "it doesn't account for all the water associated with casein in milk. Another problem is the lack of definable structure. That's a cop-out. Let's just say the theory doesn't hold water."

Instead of ambiguous and haphazard molecular clusters surrounded by water, the scientists found that casein particles have distinctive and predictable structures permeated by unbound water.

Their findings, they say, could provide a basis for scientifically modifying and eventually automating the initial and often costliest stage of cheese production.

"This is the step when enzymes are added to milk to make the casein break down, lose water, and clot," Farrell explains. The milk then coagulates into a soft, puddinglike gel, called curd, which is separated from the rest of the

Soy Oil Has All the Moxie of Fish Oil

The human body rapidly converts a component in soybean oil into the same kinds of fatty acids found in fish oil, an Agricultural Research Service scientist has discovered.

In a preliminary study, two men consumed chemically labeled linolenic acid from soybean oil. Within a few hours, their bodies converted it into the same omega-3 fatty acids as those in fish and marine oil, says chemist Edward A. Emken who led the study conducted with help from St. Francis Medical Center in Peoria, Illinois.

He says the finding is the first direct evidence confirming scientists' earlier suspicions that the conversion occurs. It could mean, he adds, that healthy people on a typical U.S. diet may have to look no farther than the dressing they put on their salad for the same benefits that some currently seek from fish oil capsules.

Capsules of fish and marine oils, touted by advertisers and debated by nutritionists, have become popular with many consumers hoping to ease or prevent conditions ranging from heart disease to rheumatoid arthritis.

Emken says that while more data is needed to resolve this controversy, "our study is the first to show that linolenic acid is rapidly converted to omega-3 fatty acids found in marine oil. The study also provides direction for further research to learn which dietary fatty acids, including those in fish and soybeans, best meet human nutritional needs."

In the study—of four healthy male volunteers—researchers used a technique called stable isotope labeling that "enabled us to see precisely what was happening," he says.

After two of the men drank milk shakes with labeled linolenic acid, blood samples from all four were taken periodically for 3 days. In samples from the two who drank the shakes, converted fatty acids began showing up within a couple of hours and peaked in about 6.

He points out that linolenic omega-3 fatty acids have a shorter chain of carbon atoms and fewer hydrogen atoms than those in fish oil. But, he says, the study indicates that the human body converts the linolenic omega-3's into fish-oil types by lengthening the carbon chains and removing some hydrogen.

In the average U.S. diet, soybean oil—the only major domestic vegetable oil containing much linolenic acid—makes up 69 percent of the vegetable fats. These fats are in foods such as shortening, margarine, and cooking oil as well as salad dressing.

"A recent U.S. Surgeon General report advises that most Americans should reduce their fat intake," Emken says, "so people shouldn't go overboard and consume so much soybean oil that they increase their total fat intake."—By **Ben Hardin,** ARS.

Edward A. Emken is in USDA-ARS Vegetable Oils Research, Northern Regional Research Center, 1815 North University St., Peoria, IL 61604 (309) 685-4011, ext. 280. ♦

milk to become cheese. The type and quality of the curd, and therefore the cheese, ultimately depend on the degree of clotting of the casein.

Cheesemakers control the clotting by adding calcium chloride to the curd as it forms.

"You have to continuously monitor the thickness and texture of the curd," says Farrell. "You might even have to take some of the stuff out and feel it with your fingers to know how much calcium to add. It's a real art—but knowing how water and casein interact at the molecular level can make it a science."

To gain that knowledge, Farrell, Pessen, and Kumosinski examined casein molecules with small-angle X-ray scattering and nuclear magnetic resonance. It was the first time in the study of proteins that these two state-of-the-art techniques were combined.

"We found that casein was truly unique," says Pessen. "Its molecular structure is very loose, and it contains at least 10 times as much water as any other protein."

The X-ray scattering showed that large casein particles consist of small subparticles arranged in a uniform way with vast amounts of space between them. Nuclear magnetic resonance data showed that water moves through these spaces, as if they were channels, giving the enzymes ready access to all of the casein.

"Our findings totally contradict the idea of water being restricted to the outside of each particle with strands of casein poking through like seaweed," says Kumosinski.

Under the previous theory, only the strands were dissolved by enzymes. This resulted in sticky patches, like islands, scattered about the watery surface. Presumably, the patches were hydrophobic (didn't like water) and would bond with patches on other particles just to keep the water away.

"Now that we've seen how water, enzymes, and salts like calcium chloride are able to move through the entire casein structure," Kumosinski says, "we can quantify a lot of what the cheesemaker has to know from experience. We can mathematically predict casein clotting times, for example, and determine optimum conditions for the clotting process."

Will this replace the art of cheesemaking?

Farrell laughs.

"I doubt it," he says. "We're simply giving the artist a scientific helping hand."—By **Steve Miller,** ARS

Thomas F. Kumosinski, Helmut Pessen, and Harold M. Farrell are at the USDA-ARS Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118 (215) 233-6595. ♦

Forum

(Continued From Page 2)

developed, and similar programs for other physical and chemical properties of pesticides will follow.

None of these expert systems will judge the accuracy of the data, but they will assess the work on which the data is based. Through answers to direct questions about laboratory procedures and techniques, the programs will determine how well the research was done and rate the reliability of the data accordingly. If the published research isn't well-documented, or if the procedures and techniques aren't appropriate, the ratings suffer.

The expert system for selenium data, called SELEX, asks 100 questions about the research methods used for each food being studied. Five aspects of the research are covered. These include the number of food samples, how the samples were obtained, how they were handled, the analytical methods used, and analytical quality control measures.

The questions all require only a yes, no, or number. They are grouped by research procedure and technique considerations and arranged by order of importance. A typical part of the sequence for sample handling reads as follows: Was homogenization of the sample required? Was the sample moisture level documented? Was the moisture level of the sample appropriate?

Once the Q-&-A sections of SELEX are completed, a program for statistical analysis takes over. Responses to the questions are variously weighted and combined into a quality index for each aspect of the research. An overall "confidence code" or reliability rating is then computed for the actual data on selenium.

Expert systems for evaluating pesticide data will operate similarly. We will probably refer to all of them as Data Quality Assessment Systems, or DAQUAS.

DAQUAS ratings can augment computer models. They can also be published as caveats with journal articles. And they can alert scientists to questionable data that might otherwise be incorporated into their own research.

With such systems, however, the end product—the data rating—is not always as useful as the means for achieving it. Since the systems work by assessing research methods and procedures, their best use might be in the laboratory while the research is in progress. After completing their research, scientists can run the system again to make sure their manuscripts touch all the procedural bases.

As for the peer review process, we submit that DAQUAS can make it more vigorous, more thorough, and more objective. Editors of scientific journals can also use the systems to help decide whether or not a paper should be published. Indeed, if questionable data has gone this far, the journal editors and DAQUAS can still keep it from being accepted as fact.

Stephen R. Heller
Douglas W. Bigwood
USDA-ARS Systems Research

Goodbye, Height-Weight Tables?

There's new hope for people who dread height-weight tables—those insurance company formulations of how much you should weigh, based on your height and frame size.

The troublesome tables might someday be edged out by handy charts of fat-to-lean ratios, says research physiologist Marta Van Loan at ARS' Western Human Nutrition Research Center in San Francisco.

And those ratios may prove to be a fairer and more accurate gauge of fitness. Because muscle is heavier than fat, the trim, well-muscled person who works out regularly may show up as overweight on the conventional height-weight tables. If measured for fat-and-lean, however, every ounce of muscle boosts the fitness rating.

But updated fat-to-lean ratios for people of different bone structures, ages, and ethnic groups must be compiled before this approach to measuring fitness can become as well known and as widely used as the traditional tables, Van Loan says. Current standards for "healthy" fat-to-lean ratios are based only on measurements of white men and women ages 20-35.

To fill in the missing information about other people's ideal ratios, Van Loan is experimenting with several different electronic instruments for measuring body fat, ranging from portable, hand-held devices to the large TOBEC machine, originally designed for the meat industry to measure fat in pigs and other animals. Researchers at Columbia University's School of Medicine in New York City were the first to come up with the idea of using the meat-monitoring machine to measure people. ARS researchers worked with the manufacturer to adapt the instrument for that use.

Van Loan says body fat measurements taken with TOBEC (for "Total Body Electrical Conductivity") are quick, reliable, painless, and just as accurate as readings from the long-established but cumbersome process of underwater weighing.

TOBEC distinguishes fat from lean by emitting the same kind of harmless electrical field that is used in airport metal detectors. Salts of lean tissue—found in muscle and water—easily conduct this electricity. Fat doesn't.

Because it only takes a few seconds to move someone though TOBEC's field, the experimental machine offers researchers an unparalleled opportunity to quickly take fat measurements of large numbers of people, and to use these new measurements to develop updated fat-to-lean ratios.—By **Marcia Wood,** ARS.

Marta Van Loan is at the USDA-ARS Western Human Nutrition Research Center, P.O. Box 29997, Presidio of San Francisco, CA 94129 (415) 556-5729. ♦

Walnut Gets a New Gene

Walnut's tiny white embryos hold the key to moving useful new genes into that species and others.

Research horticulturist Gale H. McGranahan with the Agricultural Research Service and colleague Abhaya M. Dandekar of the University of California at Davis recently transferred a foreign gene into specially cultured walnut embryos—a laboratory first.

Two of six dozen embryos that McGranahan and Dandekar used in the experiment have passed on a gene from a bacterium, *Agrobacterium tumefaciens,* into new embryos and plantlets.

GALE MCGRANAHAN

Walnut's tiny white embryos hold the key to moving useful new genes into that species and others. (88BW2143)

They say the embryo route may be the best-yet approach for moving new genes into other crops as well, including pecan, almond, grape, cherry, and peach. Like walnut, those crops can be induced to produce successive generations of embryos in the laboratory—a phenomenon known as repetitive somatic embryogenesis.

California's Walnut Marketing Board funded part of the research.

Although the marker gene that the two scientists transferred doesn't confer useful traits, the accomplishment paves the way for giving new genes that are valuable to the walnut species. Two transfers of top research priority: A gene that gives resistance to codling moth and genetic material that may protect walnut from blackline, a disease that weakens and kills walnut trees.

Here's how the scientists moved the marker gene: The first step was to bathe the embryos in a special *A. tumefaciens* bacterium. (Other scientists had previously genetically engineered the bacterium to contain a gene that gives resistance to an antibiotic.) The bath gave the

bacterium and the gene the chance to move into the embryos.

The embryos were allowed to multiply, and the new-generation embryos were exposed to the antibiotic. The survivors—those that had taken up the resistance gene from the bacterium—went on to produce another generation of embryos. But embryos that hadn't taken up the new gene stopped producing new embryos.

The scientists moved the marker gene into walnut in only 9 months—a feat possible only with genetic engineering, McGranahan says. "Before modern biotechnology, you couldn't take a gene from one form of life, such as a bacterium, and move it into another, such as a tree. With traditional breeding, walnut could only be crossed with other members of the same species or a closely related species. And using conventional breeding to move just a single gene into a commercial variety of walnut could easily take a lifetime."—By **Marcia Wood,** ARS.

Gale H. McGranahan is with USDA-ARS Crops Pathology and Genetics, 3116 Wickson Hall, University of California, Davis, CA 95616 (916) 752-0113. ♦

Patents

Cucumber Pickles: More Crunch and Less Salt

Forget building a better mousetrap—researcher Henry P. Fleming is on to a better pickle.

Fleming is head of a team of scientists that has developed a microorganism that would allow cucumber pickling with less salt and is less likely to cause softening or bloat during fermentation.

During the past 6 years, the research team, which included microbiologist Mark A. Daeschel and biochemist Roger F. McFeeters, has chemically mutated, selected, and improved strains of *Lactobacillus plantarum* at the Agricultural Research Service's Food Science Laboratory in Raleigh, North Carolina.

L. plantarum is the bacterium that converts the sugar in cucumbers to lactic acid. The conversion is what gives pickles their characteristic sour taste. The mutated bacterium's major difference is that it cannot produce carbon dioxide gas from malic acid during fermentation of cucumbers. Carbon dioxide causes the formation of gas pockets, called bloater damage, in cucumbers, which destroys the desirable crunch of premium pickles.

The ARS patent on the organism includes a method for identifying and selecting non-gas-producing *lactobacilli.*

"It is a pretty straightforward method, depending on a simple color change to identify strains that can or cannot produce carbon dioxide," Fleming says.

ROB FLYNN

Henry Fleming, ARS food technologist, at top of closed-type pickling tank. The tank is filled and emptied through the 30-inch-diameter port. (0686X750-24)

Milwaukee-based Chr. Hansen Laboratories, Inc., was recently licensed under a research agreement between ARS and Pickle Packers International, to find the best way to grow commercial quantities of the nongas strains.

Currently, to prevent bloater damage, the pickle industry purges carbon dioxide by bubbling nitrogen gas through fermenting tanks, a procedure introduced to the industry about 15 years ago by a previous ARS research team of which Fleming was a member. Before the purging era, it was necessary for tanks to be open to let carbon dioxide escape to reduce bloating.

Fermenting tanks are also open-topped—exposed to sunlight—to inhibit molds, yeasts, and bacteria on the brine surface that can spoil pickles.

But open tanks require addition of large amounts of salt to guard against contamination by rain water and foreign material.

Introducing nongas strains would make closed-top tanks more practical, particularly if purging is not needed to remove carbon dioxide. This would allow use of lower salt concentrations during fermentation of cucumbers.

Fleming's team, which also includes North Carolina State University engineer E.G. Humphries, has designed a closed-top fermentation tank that is also being tested at an industry-supported pickle plant in Mount Olive, North Carolina.

For technical information, contact Henry P. Fleming, USDA-ARS Food Science Research Lab, North Carolina State University, Raleigh, NC 27695 (919) 737-2979.—By **Kim Kaplan,** ARS.

Patent No. 4,666,849, *"Lactic Acid Bacteria Which Do Not Decarboxylate Malic Acid and Fermentation Therewith."* ♦

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